FORM PTO-1390 (Modified) (REV 11-98) U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

Rec'd PCT/PTO 20 JAN 2000

ATTORNEY'S DOCKET NUMBER
2727-99J

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR 1.5)
09/463209

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/EP98/04510	21 July 1998	21 July 1997

TITLE OF INVENTION
NUCLEIC ACID MOLECULE, TEST KIT AND USE

APPLICANT(S) FOR DO/EO/US
Kornelia Berghof, Alexander Gasch, Freimut Wilborn and Arndt Rolfs

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☐ This is an express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4. ☒ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. ☒ A copy of the International Application as filed (35 U.S.C. 371 (c) (2))
   a. ☒ is transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☐ has been transmitted by the International Bureau.
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☐ A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. ☐ A copy of the International Search Report (PCT/ISA/210).
8. ☐ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371 (c)(3))
   a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau)
   b. ☐ have been transmitted by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☐ have not been made and will not be made.
9. ☐ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
10. ☐ An oath or declaration of the inventor(s) (35 U.S.C. 371 (c)(4)).
11. ☐ A copy of the International Preliminary Examination Report (PCT/IPEA/409).
12. ☐ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S C. 371 (c)(5)).

Items 13 to 20 below concern document(s) or information included:

13. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
14. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
15. ☐ A **FIRST** preliminary amendment.
16. ☐ A **SECOND** or **SUBSEQUENT** preliminary amendment.
17. ☐ A substitute specification.
18. ☐ A change of power of attorney and/or address letter.
19. ☐ Certificate of Mailing by Express Mail
20. ☒ Other items or information:

Amendments to Claims Under Article 34 (in German)
Declaration (unsigned)
Cover Sheet of WIPO Publication

PCTUS1/REV03

Express Mail No. EJ339413108US

420 Rec'd PCT/PTO 20 JAN 2000

U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR 1.5)	INTERNATIONAL APPLICATION NO.	ATTORNEY'S DOCKET NUMBER
09/463209	PCT/EP98/04510	2727-99J

21. The following fees are submitted:.

BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)) :

		CALCULATIONS PTO USE ONLY
☐	Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2) paid to USPTO and International Search Report not prepared by the EPO or JPO	$970.00
☒	International preliminary examination fee (37 CFR 1.482) not paid to USPTO but Internation Search Report prepared by the EPO or JPO	$840.00
☐	International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO	$690.00
☐	International preliminary examination fee paid to USPTO (37 CFR 1.482) but all claims did not satisfy provisions of PCT Article 33(1)-(4)	$670.00
☐	International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(1)-(4)	$96.00

ENTER APPROPRIATE BASIC FEE AMOUNT =	$840.00	
Surcharge of **$130.00** for furnishing the oath or declaration later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492 (e)).	$0.00	

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
Total claims	- 20 =	0	x $18.00	$0.00	
Independent claims	- 3 =	0	x $78.00	$0.00	
Multiple Dependent Claims **(check if applicable)**.			☐	$0.00	

TOTAL OF ABOVE CALCULATIONS =	$840.00	
Reduction of 1/2 for filing by small entity, if applicable. Verified Small Entity Statement must also be filed (Note 37 CFR 1.9, 1.27, 1.28) **(check if applicable)**. ☐	$0.00	
SUBTOTAL =	$840.00	
Processing fee of **$130.00** for furnishing the English translation later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492 (f)). +	$0.00	
TOTAL NATIONAL FEE =	$840.00	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31) **(check if applicable)**. ☐	$0.00	
TOTAL FEES ENCLOSED =	$840.00	
Amount to be: refunded	$	
charged	$	

☐ A check in the amount of to cover the above fees is enclosed.

☒ Please charge my Deposit Account No. **501145** in the amount of **$840.00** to cover the above fees
A duplicate copy of this sheet is enclosed. (Order No. 2727-99J)

☒ The Commissioner is hereby authorized to charge any fees which may be required, or credit any overpayment to Deposit Account No. **501145** A duplicate copy of this sheet is enclosed.
(Order No. 2727-99J)

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

Ronald R. Santucci
Pitney, Hardin, Kipp & Szuch, LLP
711 Third Avenue, 20th Floor
New York, New York 10017

(212)687-6000

SIGNATURE

Ronald R. Santucci
NAME

28,988
REGISTRATION NUMBER

January 20, 2000
DATE

2727-99J

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants: Kornelia Berghof, Alexander Gasch, Charles Mason-Brown, Freimut Wilborn, Arndt Rolfs

Serial No.: 09/463,209

International Appln. No.: PCT/EP98/04510

International Filing Date: July 21, 1998

Priority Date Claimed: July 21, 1997

For: NUCLEIC ACID MOLECULE, KIT AND USE

PRELIMINARY AMENDMENT

Box PCT
Commissioner for Patents
Washington, D.C. 20231
Attn: DO/EO/US

S I R:

Preliminary to examination of the above-identified application kindly amend the application as follows:

In the Claims:

In claim 9, lines 1, 3-4, 6-7, 9-10, 12-13, and 15, each time kindly delete "any one of the preceding claims" and substitute therefor --claim 1--;

In claim 11, line 1, kindly delete "any one of the preceding claims" and substitute therefor --claim 1--;

In claim 12, line 1, kindly delete "any one of the preceding claims" and substitute therefor --claim 1--;

In claim 14, line 1, kindly delete "or claim 13";

In claim 15, lines 1-2, kindly delete "any one of the preceding claims" and substitute therefor --claim 1--;

In claim 18, line 1, kindly delete "any one of claims 15 to 17" and substitute therefor --claim 15--;

In claim 20, line 1, kindly delete "any one of claims 15 to 19" and substitute therefor --claim 15--;

In claim 30, lines 5, 7-8, 10-11 and 13, each time kindly delete "any one of claims 22 to 29" and substitute therefor --claim 22--;

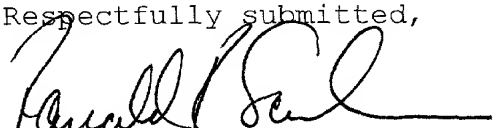
In claim 32, lines 1-2, kindly delete "any one of claims 22 to 30" and substitute therefor --claim 22--;

In claim 33, lines 1-2, kindly delete "any one of claims 22 to 32" and substitute therefor --claim 22--.

REMARKS

The specification of the above-identified application has been amended to remove all multiple dependencies. (Please note that the claims being amended are those previously amended according to Article 34 of Chapter II PCT.) No new matter has been added. Accordingly, an early examination of the application is respectfully requested.

Respectfully submitted,



Ronald R. Santucci
Registration No. 28,988

Pitney, Hardin, Kipp & Szuch, LLP
711 Third Avenue, 20th Floor
New York, New York 10017
212-687-6000

VERIFIED STATEMENT (DECLARATION) CLAIMING SMALL ENTITY STATUS (37 CFR 1.9(f) AND 1.27 (c)) - SMALL BUSINESS CONCERN				Docket No. 2727-291
Serial No. 09/463,209		Filing Date	Patent No.	Issue Date

Applicant, Patentee: Kornelia Berghof, Alexander Gasch, Charles Mason-Brown, Freimut Wilborn, Arndt Rolfs

Invention: NUCLEIC ACID MOLECULE, TEST KIT AND USE

I hereby declare that I am:

- ☐ the owner of the small business concern identified below:
- ☒ an official of the small business concern empowered to act on behalf of the concern identified below:

NAME OF CONCERN: Biotecon Gesellschaft fuer Biotechnologische Entwicklung und Consulting mbH

ADDRESS OF CONCERN: Teppler Weg 33, D-10589, Berlin, Germany

I hereby declare that the above-identified small business concern qualifies as a small business concern as defined in 13 CFR 121.3-18, and reproduced in 37 CFR 1.9(d), for purposes of paying reduced fees under Section 41(a) and (b) of Title 35, United States Code, in that the number of employees of the concern, including those of its affiliates, does not exceed 500 persons. For purposes of this statement, (1) the number of employees of the business concern is the average over the previous fiscal year of the concern of the persons employed on a full-time, part-time or temporary basis during each of the pay periods of the fiscal year, and (2) concerns are affiliates of each other when either, directly or indirectly, one concern controls or has the power to control the other, or a third party or parties controls or has the power to control both.

I hereby declare that rights under contract or law have been conveyed to and remain with the small business concern identified above with regard to the above identified invention described in:

- ☐ the specification filed herewith with title as listed above.
- ☒ the application identified above.
- ☐ the patent identified above.

If the rights held by the above-identified small business concern are not exclusive, each individual, concern or organization having rights to the invention is listed on the next page and no rights to the invention are held by any person, other than the inventor, who could not qualify as an independent inventor under 37 CFR 1.9(c) or by any concern which would not qualify as a small business concern under 37 CFR 1.9(d) or a nonprofit organization under 37 CFR 1.9(e).

Each person, concern or organization to which I have assigned, granted, conveyed, or licensed or am under an obligation under contract or law to assign, grant, convey, or license any rights in the invention is listed below:

☒ no such person, concern or organization exists.
☐ each such person, concern or organization is listed below.

FULL NAME
ADDRESS

☐ Individual ☐ Small Business Concern ☐ Nonprofit Organization

FULL NAME
ADDRESS

☐ Individual ☐ Small Business Concern ☐ Nonprofit Organization

FULL NAME
ADDRESS

☐ Individual ☐ Small Business Concern ☐ Nonprofit Organization

FULL NAME
ADDRESS

☐ Individual ☐ Small Business Concern ☐ Nonprofit Organization

Separate verified statements are required from each named person, concern or organization having rights to the invention averring to their status as small entities. (37 CFR 1.27)

I acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate. (37 CFR 1.28(b))

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

NAME OF PERSON SIGNING: K. Berghof

TITLE OF PERSON SIGNING OTHER THAN OWNER: Director

ADDRESS OF PERSON SIGNING: Tegler Weg 33, D-10589, Berlin, Germany

SIGNATURE: Berghof DATE: 16.3.2000

(K. Berghof)

Nucleic acid molecule, kit and use

General background of the invention

A bacterium of great clinical relevance is the gram-positive bacterium *Staphylococcus aureus*. It is one of the most frequent causes of nosocomial infections (infections transmitted in hospital), the spread of which is difficult to control because of the occurrence of various forms of antibiotic resistance (for example, resistance to methicillin and vancomycin). *Staphylococcus aureus* is also one of the most frequent causes of food poisoning, which is generally caused by enterotoxins. The frequent occurrence of *Staphylococcus aureus* in foodstuff products therefore requires a regular investigation of potentially contaminated products. Conventional microbiological methods for the detection of *Staphylococcus aureus* are very time-consuming (at least 4 days). There is therefore a great need for alternative methods, by means of which the presence of *Staphylococcus aureus* can be diagnosed rapidly and reliably in the course of the production process (from the raw materials to the finished product).

A number of new methods for routine use in the detection of microorganisms have been developed in recent years. These include immunological methods based on the use of polyvalent or monoclonal antibodies, and methods in which nucleic acid probes are used for detection by means of hybridisation to organism-specific nucleic acids. Further methods described are those based on amplification of a specific nucleic acid, with or without a subsequent confirmation reaction by nucleic acid hybridisation.

Methods used for the amplification of nucleic acids are, for example, polymerase chain reaction (PCR) [US Patents 4,683,195; 4,683,202; and 4,965,188], ligase chain reaction [WO Publication 89/09835], "self-sustained sequence replication" [EP 329 822], the "transcription based amplification system" [EP 310 229] and the Qβ RNA-replicase system [US Patent 4,957,858].

The nucleic-acid-based methods mentioned are so sensitive that, unlike in conventional microbiological methods, the tedious process of increasing the quantity of the microorganism to be detected from the sample to be investigated is unnecessary. A test for the presence or absence of the microorganism in question is therefore generally concluded within one working day when using the nucleic-acid-based methods mentioned. This constitutes a considerable time saving, in particular when several days to several weeks are needed for detection by conventional methods.

A number of rapid tests have recently been developed by means of which the presence of *Staphylococcus aureus* can be significantly shortened. These include coagulase tests, various agglutination tests, TNase tests (enzyme activity, monoclonal antibodies), DNA hybridisation tests and PCR tests [for an overview, see BRAKSTAD and MAELAND, (1995), APMIS 103, 209-218]. PCR tests are superior to the other methods in terms of speed and specificity. The PCR tests described hitherto for the specific detection of the *Staphylococcus aureus* species are based on the gene for thermostable nuclease [*nuc*, Brakstad *et al.*, (1992), J.

Clin. Microbiol. 30, 1654-1660] or on a regulator gene essential for methicillin resistance (*femA*), which is specific for *Staphylococcus aureus* [Ünal *et al.*, (1992), J. Clin. Microbiol. 30, 1685-1691; Vannuffel *et al.*, (1995), J. Clin. Microbiol., 33, 2864-2867]. Using those PCR systems, it has been possible to detect all the investigated species of *Staphylococcus aureus*. It is unclear, however, whether it is also possible to detect coagulase-negative strains of the species *Staphylococcus aureus* using those PCR systems.

The aim of the present invention was to establish a PCR system, the use of which as primers and/or probes ensures as complete detection as possible of all the representatives of the species *Staphylococcus aureus*.

Depending upon the size of the group of microorganisms to be detected and on the evolutionary relationship of microorganisms to be delimited (not to be detected), detection based on differential DNA sequences requires very comprehensive preliminary work in order to find suitable DNA sequences having the desired specificity in each case. The invention described herein relates to DNA sequences by means of which the rapid detection of bacteria of the genus *Staphylococcus*, especially of *Staphylococcus aureus*, is possible.

Description of the invention

To detect specific microorganisms by means of nucleic acid hybridisation or amplification, organism-specific oligonucleotides are used. **Organism-specific oligonucleotides**

are nucleic acids, from 10 to 250 bases long (preferably from 15 to 30 bases long), the base sequence of which is characteristic for a specific microorganism or a group of microorganisms. Hybridisation to DNA or amplification of DNA using those organism-specific oligonucleotides (for example as primers or probes) in the above-mentioned methods can be effected, under suitable reaction conditions, only when the DNA of the microorganisms to be detected is present.

Prokaryotic ribosomes generally contain three distinct nucleic acid components, generally known as 5S, 16S and 23S rRNA (ribosomal ribonucleic acid). The genetic information for those ribonucleic acids (rDNA) is typically arranged in the genome in the form of tandems. The typical organisation of such a unit is 16S-23S-5S, the three genes being separated from one another by short hypervariable intergenic regions. A plurality of units are present in the genome, it being possible for the number of repeating units to vary in different bacteria. The high degree of conservation of the DNA sequence in the region of the 16S rDNA, the 23S rDNA and the 5S rDNA throughout the entire bacterial kingdom makes it possible to design **non-specific oligonucleotides** even without precise knowledge of the DNA sequences of the microorganisms to be investigated. Such non-specific oligonucleotides are characteristic for a relatively large, generally phylogenetically related group of microorganisms. By using those non-specific oligonucleotides, the person skilled in the art will be able, for example following appropriate preliminary tests by DNA amplification by means of PCR, to isolate rDNA fragments, for example of the 23S/5S intergenic region, of any desired

microorganism. By DNA sequencing, it is then possible to determine the sequence of the hypervariable intergenic regions of the microorganism in question.

DNA sequencing of the 23S/5S intergenic region of as large as possible a number of bacteria **to be detected** (for example of various *Staphylococcus* species) on the one hand and a subsequent comparison of those DNA sequences on the other hand make it possible to locate DNA regions that are not modified or are modified only insignificantly in the group under investigation (e.g. all *Staphylococcus* species).

DNA sequencing of the 23S/5S intergenic region of as large as possible a number of bacteria **that are not to be detected** (for example all bacteria not belonging to the genus *Staphylococcus*) on the one hand and a subsequent comparison of those DNA sequences with the sequence of the bacteria to be detected (e.g. various *Staphylococcus* species) on the other hand make it possible to locate DNA sequences that are characteristic for the bacteria to be detected (e.g. all *Staphylococcus* species). From those DNA sequences it is possible in turn to derive oligonucleotides that can be used as primers and/or probes in nucleic-acid-based methods, with the aim of detecting specifically the group of bacteria in question (e.g. all species of the genus *Staphylococcus*).

The organism-specific oligonucleotides described in the present invention for the detection of bacteria of the genus *Staphylococcus*, especially bacteria of the species *Staphylococcus aureus*, correspond to the 23S/5S intergenic

region and the directly adjacent region of the 23S rDNA. The DNA sequence in that region was determined for a large number of bacteria. Following precise sequence comparisons, organism-specific nucleic acid sequences were determined, from which it is possible to derive primers and/or probes for use in a species- or genus-specific detection method.

The problem underlying the invention is solved according to an embodiment by a nucleic acid molecule that hybridises selectively to RNA or DNA of a group of bacteria of the genus *Staphylococcus*, characterised in that it contains at least 10 successive nucleotides of the region from -113 to +58 relative to the 3'-end of the 23S rDNA of a *Staphylococcus* isolate or their complementary nucleotides. The purpose of the selective hybridisation according to the invention is to detect the mentioned group of bacteria of the genus *Staphylococcus*.

A further embodiment of the invention relates to a nucleic acid molecule that hybridises selectively to RNA or DNA of a group of bacteria of the genus *Staphylococcus*, characterised in that it contains at least 10 successive nucleotides of the region from -113 to +58 relative to the 3'-end of the 23S rDNA of *Staphylococcus aureus* (ATCC 6538) or their complementary nucleotides.

A further embodiment of the invention relates to a nucleic acid molecule that hybridises selectively to RNA or DNA of a group of bacteria of the genus *Staphylococcus*, characterised in that it contains at least 10 successive nucleotides of the region from

(i) nucleotide position 54 to 83 of SEQ ID NO 1, or
(ii) nucleotide position 100 to 166 of SEQ ID NO 1, or
(iii)the sequences complementary to (i) or (ii).

A further embodiment of the invention relates to a nucleic acid molecule for the detection of the presence or absence of bacteria belonging to a group of bacteria of the genus *Staphylococcus*, characterised in that it makes it possible by means of nucleic acid hybridisation and/or nucleic acid amplification methods under suitable reaction conditions to distinguish between bacteria to be detected and bacteria that are not to be detected and that the distinction is possible or is facilitated by a differing nucleic acid sequence at at least one base position in the region of SEQ ID NO: 1, or of its complementary sequence, in the genomic DNA and/or RNA of bacteria to be detected and bacteria that are not to be detected.

A further embodiment of the invention relates to a nucleic acid molecule for the detection of the presence or absence of bacteria belonging to a group of bacteria of the genus *Staphylococcus*, characterised in that it makes it possible by means of nucleic acid hybridisation and/or nucleic acid amplification methods under reaction conditions that are suitable or are known *per se* to distinguish between bacteria to be detected and bacteria that are not to be detected and that the distinction is possible or is facilitated by a differing nucleic acid sequence at at least one base position in
(i) the region 54 to 83 of SEQ ID NO 1, or
(ii) the region 100 to 166 of SEQ ID NO 1, or
(iii)the sequence that is complementary to (i) or (ii)

in the genomic DNA and/or RNA of bacteria to be detected and bacteria that are not to be detected.

A further embodiment of the invention relates to a nucleic acid molecule of SEQ ID NO 1 or of its complementary sequence, especially for the detection according to the invention.

A further embodiment of the invention relates to a nucleic acid molecule having a sequence that is shorter than a nucleic acid molecule according to SEQ ID NO 1, namely

(i) a sequence of the region or in the region of the nucleotide positions 54 to 83, or

(ii) a sequence of the region or in the region of the nucleotide positions 100 to 166, or

(iii) a sequence that is complementary to a sequence according to (i) or (ii).

A further embodiment of the invention relates to a nucleic acid molecule having a sequence that is shorter than a nucleic acid molecule according to SEQ ID NO 1, namely

(i) SEQ ID NO 2, or

(ii) SEQ ID NO 3, or

(iii) SEQ ID NO 4, or

(iv) the sequences complementary to (i), (ii) and (iii), respectively.

A further embodiment of the invention relates to a further or different nucleic acid molecule, characterised in that in respect of its sequence in at least 10 successive nucleotides of its nucleotide chain

(i) it is identical to a nucleic acid molecule according to any one of the preceding claims, or

(ii) it corresponds in 9 out of 10 successive nucleotides to a nucleic acid molecule according to any one of the preceding claims, or

(iii) it corresponds in 8 out of 10 successive nucleotides to a nucleic acid molecule according to any one of the preceding claims, or

(iv) it is at least 90% homologous to a nucleic acid molecule according to any one of the preceding claims.

Such a nucleic acid molecule may be characterised in that it is from 10 to 250, preferably from 15 to 30, nucleotides long.

An example of a nucleic acid molecule according to (i) is characterised by SEQ ID NO 5.

A nucleic acid molecule according to the invention may be characterised in that it is single-stranded or double-stranded.

A nucleic acid molecule according to the invention may be characterised in that it is present

(i) as DNA, or

(ii) as RNA corresponding to (i), or

(iii) as PNA (see below),

the nucleic acid molecule, where appropriate, being modified in a manner known *per se* for analytical detection methods, especially methods based on hybridisation and/or amplification.

Such a nucleic acid molecule may be characterised in that the nucleic acid molecule is modified by the replacement of up to 10% of the nucleotides, especially 1 or 2 nucleotides, by analogous components known *per se* for probes and/or primers, especially by nucleotides that do not occur naturally in bacteria.

A nucleic acid molecule according to the invention may be characterised in that the nucleic acid molecule is modified or labelled or is additionally modified or labelled in that it comprises, in a manner known *per se* for analytical detection methods, one or more radioactive groups, coloured groups, fluorescent groups, groups for immobilisation on a solid phase and/or groups for an indirect or direct reaction, especially an enzymatic reaction, especially using antibodies, antigens, enzymes and/or substances having an affinity for enzymes or enzyme complexes.

A further embodiment of the invention relates to a kit for analytical detection methods, especially for the detection of bacteria of the genus *Staphylococcus*, characterised by one or more nucleic acid molecules according to the invention.

A further embodiment of the invention relates to a use of one or more nucleic acid molecules according to the invention or of a kit according to the invention for the detection of the presence or absence of bacteria belonging to a group of bacteria of the genus *Staphylococcus*.

That use may be characterised in that the group of bacteria of the genus *Staphylococcus* comprises various strains of *Staphylococcus aureus*.

That use may be characterised in that the group of bacteria of the genus *Staphylococcus* comprises exclusively *Staphylococcus aureus* strains.

Those uses may be characterised in that nucleic acid hybridisation and/or nucleic acid amplification is carried out.

That use may be characterised in that a polymerase chain reaction is carried out as nucleic acid amplification.

Those uses may be characterised in that the detection is carried out by distinguishing between the bacteria to be detected and bacteria that are not to be detected on the basis of differences in the genomic DNA and/or RNA at at least one nucleotide position in the region of a nucleic acid molecule according to the invention.

That use may be characterised in that the distinction is made on the basis of differences in the region of a nucleic acid molecule of SEQ ID NO 1.

To detect the group of microorganisms in question, nucleic acids, preferably genomic DNA, are first released from the cells contained in a sample or bacterial culture to be investigated. By means of nucleic acid hybridisation, the **direct** detection of organism-specific nucleic acid sequences in the sample to be investigated can then be

effected using the organism-specific oligonucleotides according to the invention as probe. Various methods known to the person skilled in the art are suitable for that purpose, such as, for example, "Southern blot" or "dot blot".

Preference is given, however, above all on account of the greater sensitivity, to an **indirect** detection method in which the DNA/RNA sequences sought are first amplified by means of the above-mentioned methods for the amplification of nucleic acids, preferably PCR.

The amplification of released DNA/RNA using the mentioned methods can be effected using organism-specific oligonucleotides as primers. In that case, specific amplification products are formed only when DNA/RNA of the microorganism to be detected is present. By a subsequent detection reaction using organism-specific oligonucleotides as probes, it is possible to increase the specificity of the detection method. For that subsequent detection reaction it is also possible to use non-organism-specific oligonucleotides as probes.

Alternatively, the nucleic acid amplification may be carried out in the presence of one or more non-specific oligonucleotides, so that possibly also DNA/RNA of other microorganisms that are not to be detected may be amplified. Such an amplification method is generally less specific and should therefore be backed up by a subsequent detection reaction using one or more organism-specific oligonucleotide(s) as probe(s).

Various methods are known to the person skilled in the art by means of which the amplification products that are formed in the indirect methods can be detected. These include, *inter alia*, visualisation by means of gel electrophoresis, the hybridisation of probes to immobilised reaction products [coupled to nylon or nitrocellulose filters ("Southern blots") or, for example, to beads or microtitre plates] and the hybridisation of the amplification products to immobilised probes (e.g. "reverse dot blots" or beads or microtitre plates coupled with probes).

A large number of different variants have been described by means of which organism-specific oligonucleotides (e.g. probes and primers) can be labelled or modified for the described direct or indirect detection methods. Thus, they may contain, for example, radioactive, coloured or fluorescent groups or groups that have been modified or that modify in some other manner, for example antibodies, antigens, enzymes or other substances having an affinity for enzymes or enzyme complexes. Probes and primers may be either naturally occurring or synthetically produced double-stranded or single-stranded DNA or RNA or modified forms of DNA or RNA, such as, for example, PNA (in those molecules, the sugar units are replaced by amino acids or peptides). Individual nucleotides or several nucleotides of the probes or primers may be replaced by analogous components (such as, for example, nucleotides that do not occur naturally in the target nucleic acid). In the above-mentioned indirect detection methods, detection may also be carried out using an internally labelled amplification product. This can be effected, for example, by the

integration of modified nucleoside triphosphates (e.g. coupled to digoxygenin or fluorescein) during the amplification reaction.

Suitable organism-specific oligonucleotides according to the invention are nucleic acids, preferably from 10 to 250 bases long and especially from 15 to 30 bases long, that correspond at least in a 10 base long sequence to the sequences 1 to 5 given below or to their complementary sequences. Relatively small differences (1 or 2 bases) in that 10 base long sequence are possible without loss of the requisite specificity in the amplification and/or hybridisation. The person skilled in the art will know that in the event of such relatively small differences the reaction conditions need to be altered accordingly.

The DNA sequence of *Staphylococcus aureus* (ATCC 6538) in the region of the 23S rDNA (1-113) and of the 23S/5S intergenic region (114-171) is:

(sequence 1 = SEQ ID NO 1)
TTTCCCAACTTCGGTTATAAGATCCCTCAAAGATGATGAGGTTAATAGGTTCGAGGTGG
AAGCATGGTGACATGTGGAGCTGACGAATACTAATCGATCGAAGACTTAATCAAAATAA
ATGTTTTGCGAAGCAAAATCACTTTTACTTACTATCTAGTTTTGAATGTATAA

The sequence in the region of the 23S/5S intergenic region was determined for 8 *Staphylococcus aureus* strains and for at least one representative of each of the following species: *Staphylococcus auricularis, Staphylococcus capitis, Staphylococcus cohnii, Staphylococcus epidermidis, Staphylococcus haemolyticus, Staphylococcus hominis, Staphylococcus saccharolyticus, Staphylococcus*

saprophyticus, *Staphylococcus simulans*, *Staphylococcus warneri*, *Staphylococcus xylosus*. The sequence comparisons revealed that the sequences of the different species have a high degree of homology in that region. Three regions having high sequence variability can, however, be detected, namely from nucleotide positions 64 to 73, 110 to 121 and 127 to 156. Oligonucleotides suitable for the detection of bacteria of the genus *Staphylococcus* can therefore be derived from the nucleotide sequences of the different *Staphylococcus* species in the regions 54 to 83 and 100 to 166. For example, from sequence 1 a number of oligonucleotides can be derived that span either fully or partially the variable nucleotide positions 64 to 73, 110 to 121 and 127 to 156 and that are suitable as primers or probes for the selective detection of bacteria of the species *Staphylococcus aureus*.

From sequence 1 there were derived the following oligonucleotides that are especially suitable as primers for the PCR (sequence 2 and sequence 3) and as probes (sequence 4 and sequence 5):

Oligonucleotide Sa1: (sequence 2 = SEQ ID NO 2)
5'-GTGGAAGCATGGTGACAT-3'

Oligonucleotide Sa2: (sequence 3 = SEQ ID NO 3)
5'-TAAGTAAAAGTGATTTTGCT TCG-3'

Oligonucleotide Sa3: (sequence 4 = SEQ ID NO 4)
5'-CATTTATTTTGATTAAGTCT-3'

Oligonucleotide Sa4: (sequence 5 = SEQ ID NO 5)
5'-CATTTAAATTTGATTAAGTC-3'

Example 1: Detection of bacteria of the species *Staphylococcus aureus* by polymerase chain reaction

DNA was isolated from pure cultures of the bacteria listed in Table 1 by means of standard procedures. Approximately from 10 to 100 ng of the DNA preparations were then used in the PCR in the presence of 0.6 µM oligonucleotide Sa1 (SEQ ID NO 2) and 0.6 µM oligonucleotide Sa2 (SEQ ID NO 3), 200 µM dNTP's (N = A/C/G/T mixture; Boehringer Mannheim), 2 mM $MgCl_2$, 16 mM $(NH_4)_2SO_4$, 67 mM Tris/HCl (pH 8.8), 0.01% Tween 20 and 0.03 U/µl *Taq*-polymerase (Biomaster). The PCR was carried out in a Perkin-Elmer 9600 thermocycler having the following thermoprofile:

- initial denaturing	94°C	3 min
- amplification (35 cycles)	92°C	30 sec
	60°C	10 sec
- final synthesis	72°C	5 min.

After completion of the PCR reaction, the amplification products were separated by means of agarose gel electrophoresis and visualised by staining with ethidium bromide. The expected product of 94 bp in length was observed only in the cases in which DNA of strains of the species *Staphylococcus aureus* was present (compare **Table 1**), but not in the presence of DNA of the other tested bacteria. After the end of the run, the DNA contained in the gels was transferred to nylon filters by means of standard methods and was hybridised with an equimolar mixture of the oligonucleotides Sa3 (SEQ ID NO 4) and Sa4 (SEQ ID NO 5)

biotinylated at the 5'-end in order to investigate the specificity. Hybridisation was effected in 5 x SSC, 2% blocking reagent, 0.1% lauryl sarcosine, 0.02% SDS and 20 pmol/ml of probe for 4 hours at 43°C. Washing was effected in 2 x SSC, 0.1% SDS for 1 x 5 min at room temperature and for 1 x 5 min at 43°C. Detection was effected according to standard methods by means of alkaline phosphatase conjugates (extravidin, SIGMA, # E-2636) in the presence of 5-bromo-4-chloro-3-indolyl phosphate and 4-nitro-blue tetrazolium chloride (Boehringer Mannheim).

A band was observed on the filters only in the cases in which a band had previously been visible on the agarose gel (see **Table 1**). Thus, the presence of all the fifty tested *Staphylococcus aureus* strains was detected by means of PCR and by means of hybridisation. Of the total of fifty *Staphylococcus aureus* strains tested, two are coagulase-negative (BioteCon 7030 and BioteCon 7044), which are also detected by the PCR method. On the other hand, none of the tested bacterial strains not belonging to that species was detected using that system.

Table 1: Results of the PCR amplification using oligonucleotides Sa1 and Sa2 (SEQ ID NO 2 and SEQ ID NO 3) and subsequent hybridisation using oligonucleotides Sa3/Sa4 (SEQ ID NO 4 and SEQ ID NO 5).

Species	Designation of strain	PCR	Hybridisation
Staphylococcus aureus	ATCC 6538	+	+
Staphylococcus aureus	BioteCon 194	+	+
Staphylococcus aureus	BioteCon 195	+	+
Staphylococcus aureus	BioteCon 196	+	+
Staphylococcus aureus	BioteCon 197	+	+
Staphylococcus aureus	BioteCon 494	+	+
Staphylococcus aureus	BioteCon 514	+	+
Staphylococcus aureus	BioteCon 528	+	+
Staphylococcus aureus	BioteCon 529	+	+
Staphylococcus aureus	BioteCon 530	+	+
Staphylococcus aureus	BioteCon 531	+	+
Staphylococcus aureus	BioteCon 532	+	+
Staphylococcus aureus	BioteCon 533	+	+
Staphylococcus aureus	BioteCon 534	+	+
Staphylococcus aureus	BioteCon 535	+	+
Staphylococcus aureus	BioteCon 4286	+	+
Staphylococcus aureus	BioteCon 4287	+	+
Staphylococcus aureus	BioteCon 4288	+	+
Staphylococcus aureus	BioteCon 4289	+	+
Staphylococcus aureus	ATCC 25923	+	+
Staphylococcus aureus	BioteCon 7010	+	+
Staphylococcus aureus	BioteCon 7011	+	+
Staphylococcus aureus	BioteCon 7012	+	+
Staphylococcus aureus	BioteCon 7013	+	+
Staphylococcus aureus	BioteCon 7014	+	+
Staphylococcus aureus	BioteCon 7015	+	+
Staphylococcus aureus	BioteCon 7016	+	+
Staphylococcus aureus	BioteCon 7017	+	+
Staphylococcus aureus	BioteCon 7018	+	+
Staphylococcus aureus	BioteCon 7019	+	+
Staphylococcus aureus	BioteCon 7020	+	+
Staphylococcus aureus	BioteCon 7021	+	+
Staphylococcus aureus	BioteCon 7022	+	+
Staphylococcus aureus	BioteCon 7024	+	+
Staphylococcus aureus	BioteCon 7025	+	+
Staphylococcus aureus	BioteCon 7027	+	+
Staphylococcus aureus	BioteCon 7028	+	+
Staphylococcus aureus	BioteCon 7029	+	+
Staphylococcus aureus	BioteCon 7030	+	+
Staphylococcus aureus	BioteCon 7031	+	+
Staphylococcus aureus	BioteCon 7032	+	+
Staphylococcus aureus	BioteCon 7034	+	+

Species	Designation of strain	PCR	Hybridisation
Staphylococcus aureus	BioteCon 7035	+	+
Staphylococcus aureus	BioteCon 7036	+	+
Staphylococcus aureus	BioteCon 7039	+	+
Staphylococcus aureus	BioteCon 7040	+	+
Staphylococcus aureus	BioteCon 7041	+	+
Staphylococcus aureus	BioteCon 7042	+	+
Staphylococcus aureus	BioteCon 7043	+	+
Staphylococcus aureus	BioteCon 7044	+	+
Staphylococcus arlettae	DSM 20672	-	-
Staphylococcus auricularis	DSM 20609	-	-
Staphylococcus capitis subsp. ureolyticus	BioteCon 2687	-	-
Staphylococcus caprae	DSM 20608	-	-
Staphylococcus carnosus	DSM 20501	-	-
Staphylococcus caseolyticus	DSM 20597	-	-
Staphylococcus chromogenes	DSM 20454	-	-
Staphylococcus cohnii subsp. cohnii,	DSM 20260	-	-
Staphylococcus delphini	DSM 20771	-	-
Staphylococcus epidermidis	BioteCon 515	-	-
Staphylococcus equorum	DSM 20674	-	-
Staphylococcus gallinarum	DSM 20610	-	-
Staphylococcus haemolyticus	BioteCon 2847	-	-
Staphylococcus hominis	DSM 20328	-	-
Staphylococcus hyicus	DSM 20459	-	-
Staphylococcus intermedius	DSM 20373	-	-
Staphylococcus kloosii	DSM 20676	-	-
Staphylococcus lentus	DSM 6672	-	-
Staphylococcus lugdunensis	BioteCon 2681	-	-
Staphylococcus muscae	DSM 7068	-	-
Staphylococcus saccharolyticus	DSM 20359	-	-
Staphylococcus saprophyticus	BioteCon 2685	-	-
Staphylococcus schleiferi subsp. schleiferi	DSM 4808	-	-
Staphylococcus sciuri	DSM 6671	-	-
Staphylococcus simulans	DSM 20322	-	-
Staphylococcus warneri	DSM 20036	-	-
Staphylococcus xylosus	BioteCon 2683	-	-
Bacillus cereus	DSM 31	-	n.p.
Bacillus coagulans	DSM 1	-	n.p.
Bacillus brevis	DSM 30	-	n.p.
Bacillus megaterium	DSM 32	-	n.p.
Bacillus thuringiensis	DSM 350	-	n.p.
Bacillus badius	DSM 23	-	n.p.
Bacillus sphaericus	BioteCon 3136	-	n.p.
Bacillus subtilis	ATCC 6633	-	n.p.
Bacillus circulans	BioteCon 4926	-	n.p.
Bacillus polymyxa	ATCC 8523	-	n.p.
Bacillus mycoides	BioteCon 4928	-	n.p.
Bacillus stearothermophilus	DSM 456	-	n.p.
Pseudomonas aeruginosa	BiotCon 682	-	n.p.

Species	Designation of strain	PCR	Hybridisation
Pseudomonas fluorescens	Biotecon 2439	-	n.p.
Pseudomonas cepacia	BioteCon 672	-	n.p.
Pseudomonas chlororaphis	BioteCon 1753	-	n.p.
Pseudomonas citronellolis	DSM 50332	-	n.p.
Pseudomonas mendocina	DSM 50017	-	n.p.
Pseudomonas pickettii	BioteCon 3323	-	n.p.
Pseudomonas fragi	DSM 3456	-	n.p.
Pseudomonas hydrophila	BioteCon 4883	-	n.p.
Pseudomonas putida	BioteCon 4884	-	n.p.
Pseudomonas oleovorans	DSM 1045	-	n.p.
Pseudomonas pseudoalcaligenes	DSM 50188	-	n.p.
Pseudomonas syringae	DSM 10604	-	n.p.
Pseudomonas mendocina	DSM 50017	-	n.p.
Pseudomonas corrugata	DSM 7228	-	n.p.
Lactobacillus brevis	DSM 1267	-	n.p.
Lactobacillus viridescens	BioteCon 2592	-	n.p.
Lactobacillus kefiranofaciens	DSM 5016	-	n.p.
Lactobacillus lindneri	BioteCon 2599	-	n.p.
Lactobacillus fructivorans	BioteCon 2598	-	n.p.
Lactobacillus casei	DSM 20011	-	n.p.
Lactobacillus vaginalis	DSM 5837	-	n.p.
Lactobacillus fermentum	BioteCon 2597	-	n.p.
Lactobacillus intestinalis	DSM 6629	-	n.p.
Lactobacillus bifermentans	DSM 20003	-	n.p.
Lactobacillus curvatus	DSM 20019	-	n.p.
Lactobacillus coryniformis subsp. torquens	DSM 20004	-	n.p.
Lactobacillus acidophilus	BioteCon 786	-	n.p.
Lactobacillus alimentarius	DSM 20249	-	n.p.
Lactobacillus fructosus	DSM 20349	-	n.p.
Lactobacillus malefermentans	DSM 20177	-	n.p.
Lactobacillus kefir	DSM 20485	-	n.p.
Lactobacillus salivarius subsp. salivarius	DSM 20492	-	n.p.
Lactobacillus homohiochii	DSM 20571	-	n.p.
Lactobacillus sanfrancisco	DSM 20663	-	n.p.
Lactobacillus aviarius	DSM 20655	-	n.p.
Lactobacillus ruminis	DSM 20403	-	n.p.
Leuconostoc mesenteriodes subsp. mesenteriodes	DSM 20240	-	n.p.
Leuconostoc mesenteriodes subsp. dextranicum	DSM 20071	-	n.p.
Leuconostoc mesenteriodes subsp. cremoris	DSM 20346	-	n.p.
Leuconostoc lactis	DSM 20202	-	n.p.
Leuconostoc oenos	DSM 20255	-	n.p.
Leuconostoc gelidum	DSM 5578	-	n.p.
Leuconostoc carnosum	DSM 5576	-	n.p.
Leuconostoc citreum	DSM 20188	-	n.p.
Leuconostoc paramesenteroi-des	DSM 20288	-	n.p.

Species	Designation of strain	PCR	Hybridisation
Leuconostoc fallax	DSM 20189	-	n.p.
Leuconostoc pseudomesenteroides	DSM 5625	-	n.p.
Streptococcus downei	DSM 5635	-	n.p.
Streptococcus sp.	DSM 6176	-	n.p.
Streptococcus parauberis	DSM 6631	-	n.p.
Streptococcus gordonii	DSM 6777	-	n.p.
Streptococcus sp.	DSM 20061	-	n.p.
Streptococcus equinus	DSM 20062	-	n.p.
Streptococcus salivarius subsp. thermophilus	DSM 20259	-	n.p.
Streptococcus canis	DSM 20386	-	n.p.
Streptococcus cricetus	DSM 20562	-	n.p.
Streptococcus anginosus	DSM 20563	-	n.p.
Streptococcus rattus	DSM 20564	-	n.p.
Streptococcus pneumoniae	DSM 20566	-	n.p.
Streptococcus pleomorphus	DSM 20574	-	n.p.
Streptococcus iniae	DSM 20576	-	n.p.
Streptococcus hansenii	DSM 20583	-	n.p.
Streptococcus ferus	DSM 20646	-	n.p.
Streptococcus alactolyticus	DSM 20728	-	n.p.
Streptococcus hyointestinalis	DSM 20770	-	n.p.
Pediococcus parvulus	DSM 20332	-	n.p.
Campylobacter jejuni subsp. jejuni	DSM 4688	-	n.p.
Citrobacter freundii	DSM 30040	-	n.p.
Shigella flexneri	DSM 4782	-	n.p.
Pectinatus cerevisiiphilus	DSM 2834	-	n.p.
Pediococcus inopinatus	DSM 20285	-	n.p.
Proteus mirabilis	BioteCon 4701	-	n.p.
Megasphaera cerevisiae	DSM 20461	-	n.p.
Escherichia coli	BioteCon 4949	-	n.p.

n.p.: The hybridisation was not performed.

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BioteCon GmbH

Patent claims 1 to 33
according to Art. 34 Chapter II PCT

1. Kit for the analytical detection of bacteria of the genus *Staphylococcus*, ***characterised*** by more than one nucleic acid molecule as primer and/or probe, wherein at least one of the nucleic acid molecules hybridises selectively to RNA or DNA of a group of bacteria of the genus *Staphylococcus*, wherein it contains at least 10 successive nucleotides of the region from -113 to +58 relative to the 3'-end of the 23S rDNA of a *Staphylococcus* isolate or their complementary nucleotides.

2. Kit for the analytical detection of bacteria of the genus *Staphylococcus*, ***characterised*** by more than one nucleic acid molecule as primer and/or probe, wherein at least one of the nucleic acid molecules hybridises selectively to RNA or DNA of a group of bacteria of the genus *Staphylococcus*, wherein it contains at least 10 successive nucleotides of the region from -113 to +58 relative to the 3'-end of the 23S rDNA of *Staphylococcus aureus* (ATCC 6538) or their complementary nucleotides.

3. Kit for the analytical detection of bacteria of the genus *Staphylococcus*, ***characterised*** by more than one

nucleic acid molecule as primer and/or probe, wherein at least one of the nucleic acid molecules hybridises selectively to RNA or DNA of a group of bacteria of the genus *Staphylococcus*, wherein it contains at least 10 successive nucleotides of the region from
(i) nucleotide position 54 to 83 of SEQ ID NO 1, or
(ii) nucleotide position 100 to 166 of SEQ ID NO 1, or
(iii) the sequences complementary to (i) or (ii).

4. Kit for the analytical detection of bacteria of the genus *Staphylococcus*, ***characterised*** by more than one nucleic acid molecule as primer and/or probe for the detection of the presence or absence of bacteria belonging to a group of bacteria of the genus *Staphylococcus*, wherein at least one of the nucleic acid molecules makes it possible by means of nucleic acid amplification and/or nucleic acid hybridisation methods under suitable reaction conditions to distinguish between bacteria to be detected and bacteria that are not to be detected, and wherein the distinction is possible by virtue of a differing nucleic acid sequence at at least one base position in the region of SEQ ID NO: 1, or of its complementary sequence, in the genomic DNA and/or RNA of bacteria to be detected and bacteria that are not to be detected .

5. Kit for the analytical detection of bacteria of the genus *Staphylococcus*, ***characterised*** by more than one nucleic acid molecule as primer and/or probe for the detection of the presence or absence of bacteria belonging to a group of bacteria of the genus *Staphylococcus*, wherein at least one of the nucleic acid molecules makes it possible by means of nucleic acid hybridisation and/or

nucleic acid amplification methods under reaction conditions known *per se* to distinguish between bacteria to be detected and bacteria that are not to be detected, and wherein the distinction is possible by virtue of a differing nucleic acid sequence at at least one base position in

(i) the region 54 to 83 of SEQ ID NO 1, or

(ii) the region 100 to 166 of SEQ ID NO 1, or

(iii) the sequence that is complementary to the region according to (i) or (ii)

in the genomic DNA and/or RNA of bacteria to be detected and bacteria that are not to be detected.

6. Kit according to claim 5, ***characterised*** by a nucleic acid molecule that has the SEQ ID NO 1 or its complementary sequence.

7. Kit according to claim 6, ***characterised*** by a nucleic acid molecule having a sequence that is shorter than a nucleic acid molecule according to claim 6, namely

(i) a sequence of the region or in the region of the nucleotide positions 54 to 83, or

(ii) a sequence of the region or in the region of the nucleotide positions 100 to 166, or

(iii) a sequence that is complementary to a sequence according to (i) or (ii).

8. Kit according to claim 6, ***characterised*** by a nucleic acid molecule having a sequence that is shorter than a nucleic acid molecule according to claim 6, namely

(i) SEQ ID NO 2, or

(ii) SEQ ID NO 3, or

(iii) SEQ ID NO 4, or

(iv) the sequences complementary to (i), (ii) and (iii), respectively.

9. Kit according to any one of the preceding claims, ***characterised*** by a nucleic acid molecule that differs from a nucleic acid molecule according to any one of the preceding claims but that in respect of its sequence in at least 10 successive nucleotides of its nucleotide chain

(i) is identical to the nucleic acid molecule according to any one of the preceding claims, or

(ii) corresponds in 9 out of 10 successive nucleotides to the nucleic acid molecule according to any one of the preceding claims, or

(iii) corresponds in 8 out of 10 successive nucleotides to the nucleic acid molecule according to any one of the preceding claims, or

(iv) is at least 90% homologous to the nucleic acid molecule according to any one of the preceding claims.

10. Kit according to claim 9, ***characterised*** in that the nucleic acid molecule is from 10 to 250, preferably from 15 to 30, nucleotides long, especially characterised in that it is the nucleic acid molecule having the sequence SEQ ID NO 5.

11. Kit according to any one of the preceding claims, ***characterised*** in that the nucleic acid molecule is single-stranded or double-stranded.

12. Kit according to any one of the preceding claims, ***characterised*** in that the nucleic acid molecule is present

(i) as DNA, or
(ii) as RNA corresponding to (i), or
(iii) as PNA,
the nucleic acid molecule, where appropriate, being modified in a manner known *per se* for analytical detection methods, especially methods based on hybridisation and/or amplification.

13. Kit according to claim 12, ***characterised*** in that the nucleic acid molecule is modified by the replacement of up to 10% of the nucleotides, especially 1 or 2 nucleotides, by analogous components known *per se* for probes and/or primers, especially by nucleotides that do not occur naturally in bacteria.

14. Kit according to claim 12 or claim 13, ***characterised*** in that the nucleic acid molecule is modified or labelled or is additionally modified or labelled in that it comprises, in a manner known *per se* for analytical detection methods, one or more radioactive groups, coloured groups, fluorescent groups, groups for immobilisation on a solid phase and/or groups for an indirect or direct reaction, especially an enzymatic reaction, especially using antibodies, antigens, enzymes and/or substances having an affinity for enzymes or enzyme complexes, or it comprises, in a manner known *per se* for analytical detection methods, groups that have been modified or that modify in some other manner.

15. Use of a kit according to any one of the preceding claims for the detection of the presence or absence of

bacteria belonging to a group of bacteria of the genus *Staphylococcus*.

16. Use according to claim 15, ***characterised*** in that the group of bacteria of the genus *Staphylococcus* comprises various strains of *Staphylococcus aureus*.

17. Use according to claim 16, ***characterised*** in that the group of bacteria of the genus *Staphylococcus* comprises exclusively *Staphylococcus aureus* strains.

18. Use according to any one of claims 15 to 17, ***characterised*** in that nucleic acid hybridisation and/or nucleic acid amplification is carried out.

19. Use according to claim 18, ***characterised*** in that a polymerase chain reaction is carried out as nucleic acid amplification.

20. Use according to any one of claims 15 to 19, ***characterised*** in that the detection is carried out by distinguishing between the bacteria to be detected and bacteria that are not to be detected on the basis of differences in the genomic DNA and/or RNA at at least one nucleotide position in the region of a nucleic acid molecule according to any one of claims 1 to 14.

21. Use according to claim 20, ***characterised*** in that the distinction is made on the basis of differences in the region of a nucleic acid molecule according to claim 6.

22. Nucleic acid molecule that hybridises selectively to RNA or DNA of a group of bacteria of the genus *Staphylococcus*, ***characterised*** in that it contains at least 10 successive nucleotides of the region from -113 to +58 relative to the 3'-end of the 23S rDNA of a *Staphylococcus* isolate or their complementary nucleotides, excluding a nucleic acid molecule that has a sequence according to Figures 1 to 10.

23. Nucleic acid molecule that hybridises selectively to RNA or DNA of a group of bacteria of the genus *Staphylococcus*, ***characterised*** in that it contains at least 10 successive nucleotides of the region from -113 to +58 relative to the 3'-end of the 23S rDNA of *Staphylococcus aureus* (ATCC 6538) or their complementary nucleotides, excluding a nucleic acid molecule that has a sequence according to Figures 1 to 10.

24. Nucleic acid molecule that hybridises selectively to RNA or DNA of a group of bacteria of the genus *Staphylococcus*, ***characterised*** in that it contains at least 10 successive nucleotides of the region from
(i) nucleotide position 54 to 83 of SEQ ID NO 1, or
(ii) nucleotide position 100 to 166 of SEQ ID NO 1, or
(iii) the sequences complementary to (i) or (ii),
excluding a nucleic acid molecule that has a sequence according to Figures 1 to 10.

25. Nucleic acid molecule for the detection of the presence or absence of bacteria belonging to a group of bacteria of the genus *Staphylococcus*, ***characterised*** in that it makes it possible by means of nucleic acid hybridisation

and/or nucleic acid amplification methods under suitable reaction conditions to distinguish between bacteria to be detected and bacteria that are not to be detected and that the distinction is possible by virtue of a differing nucleic acid sequence at at least one base position in the region of SEQ ID NO: 1, or of its complementary sequence, in the genomic DNA and/or RNA of bacteria to be detected and bacteria that are not to be detected, excluding a nucleic acid molecule that has a sequence according to Figures 1 to 10.

26. Nucleic acid molecule for the detection of the presence or absence of bacteria belonging to a group of bacteria of the genus *Staphylococcus*, ***characterised*** in that it makes it possible by means of nucleic acid hybridisation and/or nucleic acid amplification methods under reaction conditions known *per se* to distinguish between bacteria to be detected and bacteria that are not to be detected and that the distinction is possible by virtue of a differing nucleic acid sequence at at least one base position in
(i) the region 54 to 83 of SEQ ID NO 1, or
(ii) the region 100 to 166 of SEQ ID NO 1, or
(iii) the sequence that is complementary to (i) or (ii),
in the genomic DNA and/or RNA of bacteria to be detected and bacteria that are not to be detected, excluding a nucleic acid molecule that has a sequence according to Figures 1 to 10.

27. Nucleic acid molecule, ***characterised*** in that it has the SEQ ID NO 1 or its complementary sequence.

28. Nucleic acid molecule having a sequence that is shorter than a nucleic acid molecule according to claim 27, namely

(i) a sequence of the region or in the region of the nucleotide positions 54 to 83, or

(ii) a sequence of the region or in the region of the nucleotide positions 100 to 166, or

(iii) a sequence that is complementary to a sequence according to (i) or (ii).

29. Nucleic acid molecule having a sequence that is shorter than a nucleic acid molecule according to claim 27, namely

(i) SEQ ID NO 3, or

(ii) SEQ ID NO 4, or

(iii) the sequences complementary to (i) and (ii), respectively.

30. Nucleic acid molecule, ***characterised*** in that in respect of its sequence in at least 10 successive nucleotides of its nucleotide chain

(i) it is identical to a nucleic acid molecule according to any one of claims 22 to 29, or

(ii) it corresponds in 9 out of 10 successive nucleotides to a nucleic acid molecule according to any one of claims 22 to 29, or

(iii) it corresponds in 8 out of 10 successive nucleotides to a nucleic acid molecule according to any one of claims 22 to 29, or

(iv) it is at least 90% homologous to a nucleic acid molecule according to any one of claims 22 to 29.

31. Nucleic acid molecule, ***characterised*** in that it has the SEQ ID NO 5 or its complementary sequence.

32. Nucleic acid molecule according to any one of claims 22 to 30, ***characterised*** in that it is from 10 to 250, preferably from 15 to 30, nucleotides long.

33. Nucleic acid molecule according to any one of claims 22 to 32, ***characterised*** in that the nucleic acid molecule is single-stranded or double-stranded.

Abstract

The present invention relates to a nucleic acid molecule or molecules and to a method for the detection of bacteria of the genus *Staphylococcus*, especially *Staphylococcus aureus*. The invention relates also to a test kit or test kits for carrying out the mentioned detection methods.

Fig. 1

TAGTCACCAG ACATATGAAT GTAATTTATA CATTCAAAAC TAGATAGTAA GTAAAAGTGA 60

TTTTGCTTCG CAAAACATTT ATTTTGATTA AGTCTTCGAT CGATTAGTAT TCGTCAGCTC 120

CACATGTCAC CATGCTTCCA CCTCGAACCT ATTAACCTCA TCATCTTTGA GGGATCTTAT 180

AACCGAAGTT GGGAAATCTC ATCTTGAGGG GGGCTTCATG CTTAGATGCT TTCAGCACTT 240

ATCCCGTCCA CACATAGCTA CCCAGCTATG CCGTTGGCAC GACAACTGGT ACACCAGAGG 300

TATGTCCATC CCGGTCCTCT CGTACTAAGG ACAGCTCCTN TCAAATTTCC TACGNCCANG 360

ACGGATAGGG ACCGAACTGT TTTCACGACG GTNCTGAACC 400

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Fig. 2

TCGACTACCA TCGACGCTAA GGAGCTTAAC TTCTGTGTTC GGCATGGGAA CAGTGTGACT 60

CCTTGCTATA GTCACCAGAC ATATGAATGT AATTATACAT TCCAAACTAG ATAGTAAGTA 120

AAAGTGATTT GCTTCGCCAA ACATTTATTT TGATTAAGTC TTCGATCGAT TAGTATTCGT 180

CAGCTCCACA TGTCACCATG CTTCCANCTN GAA 213

Fig 2

ATTCGTCAGC TCCACATGTC ACCATGCTTC CACCTCGAAC CTATTAACCT CATCATCTTT 60

GAGGGATCTT ATAACCGAAG TTGGGAAATC TCATCTTGAG GGGGGTTCAT GCTAGATGCT 120

TCAGACTATC CCGTCCACAC ATGTAACCAG NATGCGTGGA CGCATGGAAC AGGGATGTCA 180

TCCG 184

Fig. 4

CCCGTGAAAG ATGATGAGGT TAATAGGTTT GAGGTGGAAG CATGGTGACA TGTGGAGCGT 60

GACGAATACG TAATTGA 77

Fig. 5

CGAATACTAA TCGATCGAAG ACTTAATCAA AATAAATGTT TTGCGACNAA

50

Fig. 6

AATCGTCGAA ACTTAATCAA AATAAATGTT TTGCGACAAA TCACTTTTAC
TTACTATCTA 60

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Fig. 7

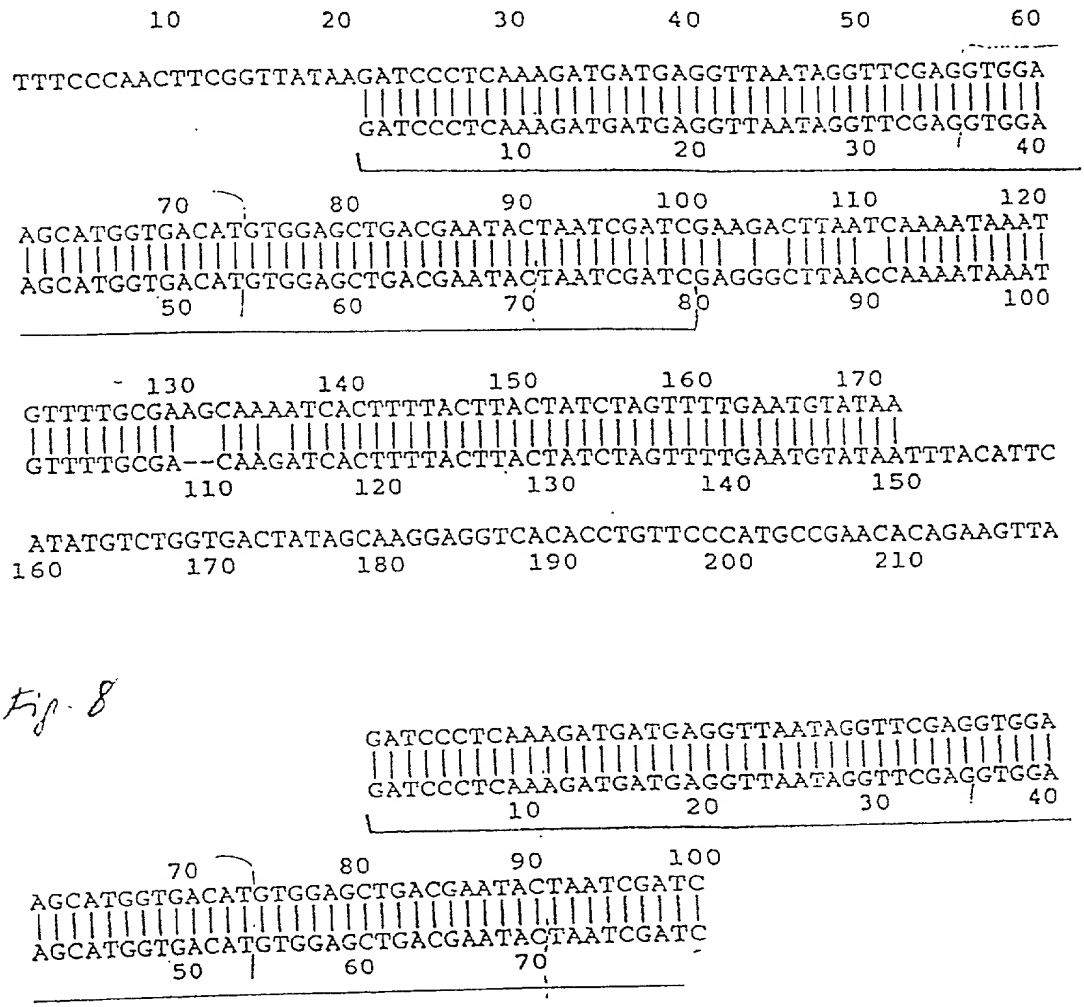

Fig. 8

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Fig. 9

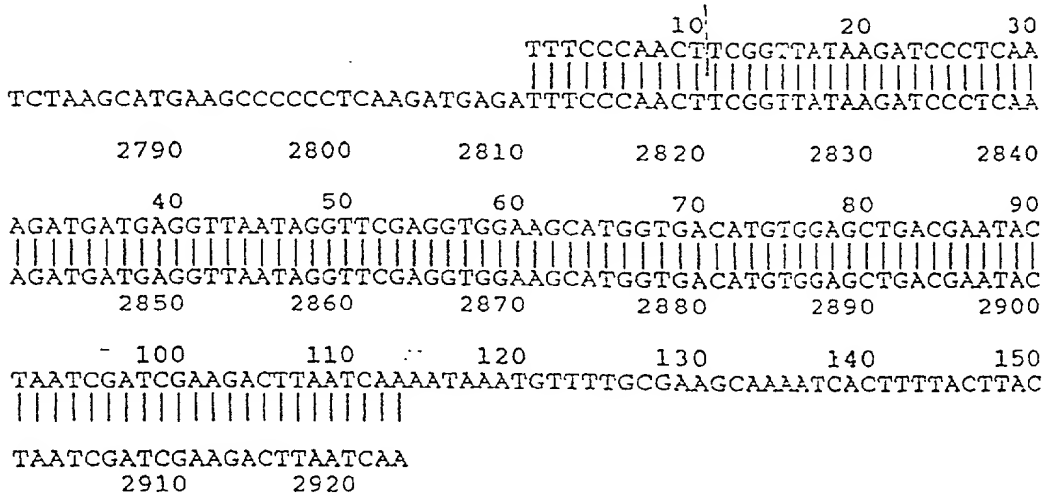
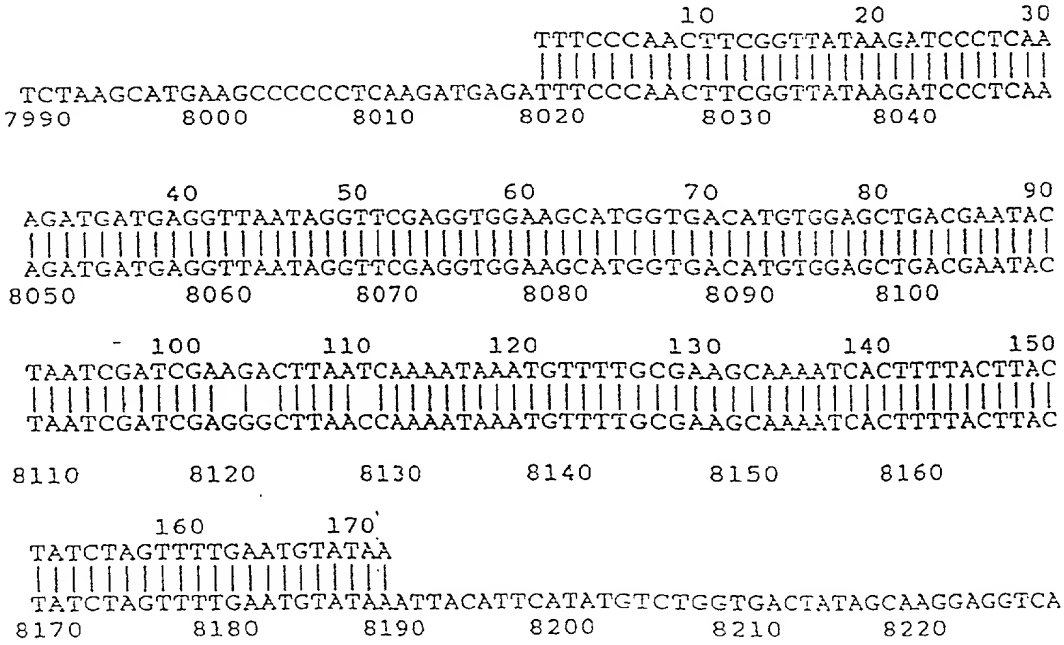


Fig. 10



(Rev. Modified) Pub.6931 FORM 1-10.1 1-75

PTO/SB/01 (5-95)
Approved for use through 9/30/98 OMB 0651-0032
Patent and Trademark Office: U.S. DEPARTMENT OF COMMERCE

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PTO/SB/01 (5-95) Rev. 6/93 — U.S. Department of Commerce, Patent and Trademark Office

DECLARATION FOR UTILITY OR DESIGN PATENT APPLICATION

☐ Declaration Submitted with Initial Filing OR ☒ Declaration Submitted after Initial Filing

Attorney Docket Number	2727-99J
First Named Inventor	Kornelia Berghof
COMPLETE IF KNOWN	
Application Number	09/463,209
Filing Date	
Group Art Unit	
Examiner Name	

As a below named inventor, I hereby declare that

my residence, post office address, and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

"Nucleic Acid Molecule, Test Kit and Use"

(Title of the Invention)

The specification of which

☐ is attached hereto

OR

☒ was filed on (MM/DD/YYYY) 07/21/1998 as United States Application Number or PCT International Application Number (USSN 09/463,209) PCT/EP98/04510 and was amended on (MM/DD/YYYY) (if applicable)

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment specifically referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in Title 37 Code of Federal Regulations, §1.56

I hereby claim foreign priority benefits under Title 35, United States Code §119 (a)-(d) or §365(b) of any foreign application(s) for patent or inventor's certificate, or §365 (a) of any PCT international application which designated at least one country other than the United States of America, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate, or of any PCT international application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application Number(s)	Country	Foreign Filing Date (MM/DD/YYYY)	Priority Not Claimed	Certified Copy Attached? YES	Certified Copy Attached? NO
197 31 292.6	Germany	07/21/1997	☐	☐	☐

☐ Additional foreign application numbers are listed on a supplemental priority sheet attached hereto:

I hereby claim the benefit under Title 35, United States Code, §119(e) of any United States provisional application(s) listed below

Application Number(s)	Filing Date (MM/DD/YYYY)

☐ Additional provisional application numbers are listed on a supplemental priority sheet attached hereto.

Burden Hour Statement: This form is estimated to take .e hours to complete. Time will vary depending upon the needs of the individual case. Any comments on the amount of time you are required to complete this form should be sent to the Chief Information Officer, Patent and Trademark Office, Washington [illegible]. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Assistant Commissioner for Patents, Washington, DC 2023[illegible]

#2534 P.002/007

Express Mail No: EK052279325US

Type a plus sign (+) inside this box → +

DECLARATION

Page 2

I hereby claim the benefit under Title 35, United States Code §120 of any United States application(s), or §365(c) of any PCT international application designating the United States of America, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT international application in the manner provided by the first paragraph of Title 35, United States Code §112, I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations §1.56 which occurred between the filing date of the prior application and the national or PCT international filing date of this application:

U.S. Parent Application Number	PCT Parent Number	Parent Filing Date (MM/DD/YYYY)	Parent Patent Number (if applicable)

☐ Additional U.S. or PCT international application numbers are listed on a supplemental priority sheet attached hereto

As a named inventor, I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith:

☐ Firm Name OR ☐ List attorney(s) and/or agent(s) Name and registration number below: Customer Number or Label

Name	Registration Number	Name	Registration Number
Joseph C. Sullivan	18,720	Ronald R. Santucci	28,988
Gerald Levy	24,419	Ronald E. Brown	32,200
		John F. Gulbin	33,180
		Richard J. Danyko	33,672

☐ Additional attorney(s) and/or agent(s) named on a supplemental sheet attached hereto.

Please direct all correspondence to: ☐ Customer Number or Label OR ☐ Fill in correspondence address below

Name	Ronald R. Santucci
Address	Pitney, Hardin, Kipp & Szuch, LLP
Address	711 Third Avenue, 20th Floor
City	New York, State NY ZIP 10017
Country	U.S.A. Telephone 212-687-6000 Fax 212-682-3484

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Name of Sole or First Inventor: ☐ A petition has been filed for this unsigned inventor

Given Name	Middle Initial	Family Name	Suffix e.g. Jr.
Kornelia		Berghof	

Inventor's Signature: Berghof Date: 21 03 00

Residence: City	State	Country	Citizenship
Berlin		Germany DEX	German

Post Office Address: Rhodelanderweg 85

Post Office Address:

City	State	Zip	Country	Appropriate Authority
Berlin		D-12355	Germany	

☐ Additional inventors are being named on supplemental sheet(s) attached hereto

DECLARATION

ADDITIONAL INVENTOR(S)
Supplemental Sheet

Name of Additional Joint Inventor, if any: ☐ A petition has been filed for this unsigned inventor

Given Name: Alexander | Family Name: Gasch | Suffix:

Inventor's Signature: [signature] | Date: 21 03 00

Residence: City: Berlin | State: | Country: Germany | Citizenship: Germa

Post Office Address: Steegerstr. 71

Post Office Address:

City: Berlin | State: | Zip: D-13359 | Country: Germany | Additional Address

Name of Additional Joint Inventor, if any: ☐ A petition has been filed for this unsigned inventor

Given Name: Charles | Family Name: Mason-Brown | Suffix:

Inventor's Signature: [signature] | Date: 21 03.00

Residence: City: Berlin | State: | Country: Germany DEX | Citizenship: Briti

Post Office Address: Wuhlischstr. 38

Post Office Address:

City: Berlin | State: | Zip: D-10245 | Country: Germany | Additional Address

Name of Additional Joint Inventor, if any: ☐ A petition has been filed for this unsigned inventor

Given Name: Freiest | Family Name: Wilborn | Suffix:

Inventor's Signature: [signature] | Date: 21 03.00

Residence: City: Berlin | State: | Country: Germany | Citizenship:

Post Office Address: Hase Kantstr. 9

Post Office Address:

City: Berlin | State: | Zip: D-14057 | Country: Germany | Additional Address

Name of Additional Joint Inventor, if any: ☐ A petition has been filed for this unsigned inventor

Given Name: Arndt | Family Name: Rolfs | Suffix:

Inventor's Signature: | Date:

Residence: City: Rostock | State: | Country: Germany | Citizenship:

Post Office Address: Gehlsheimerstr. 20

Post Office Address:

City: Rostock | State: | Zip: D-18055 | Country: Germany | Additional Address

☐ Additional inventors are being named on supplemental sheet(s) attached hereto

DECLARATION

ADDITIONAL INVENTOR(S) Supplemental Sheet

2-00 X

Name of Additional Joint Inventor, if any: ☐ A petition has been filed for this unsigned inventor

Given Name	Middle Initial	Family Name	Suffix
Alexander		Gasch	

Inventor's Signature: [signature] Date: 21 03 00

Residence: City: Berlin State: Country: Germany DEX Citizenship: German

Post Office Address: Steegerstr. 71

Post Office Address:

City: Berlin State: Zip: D-13359 Country: Germany Applicant Authority:

Name of Additional Joint Inventor, if any: ☐ A petition has been filed for this unsigned inventor

Given Name	Middle Initial	Family Name	Suffix
Charles		Mason-Brown	

X Inventor's Signature: Date:

Residence: City: Berlin State: Country: Germany Citizenship: British

Post Office Address: Wuhlischstr. 38

Post Office Address:

City: Berlin State: Zip: D-10245 Country: Germany Applicant Authority:

Name of Additional Joint Inventor, if any: ☐ A petition has been filed for this unsigned inventor

4-00 X

Given Name	Middle Initial	Family Name	Suffix
Freimut		Wilborn	

Inventor's Signature: [signature] Date: 21 03 00

Residence: City: Berlin State: Country: Germany DEX Citizenship: German

Post Office Address: Neue Kantstr. 9

Post Office Address:

City: Berlin State: Zip: D-14057 Country: Germany Applicant Authority:

Name of Additional Joint Inventor, if any: ☐ A petition has been filed for this unsigned inventor

5-00 X

Given Name	Middle Initial	Family Name	Suffix
Arndt		Rolfs	

Inventor's Signature: [signature] Date: 21-03-00

Residence: City: Rostock State: Country: Germany DEX Citizenship: German

Post Office Address: ~~Gehlsheimerstr. 20~~ Gerhart-Hauptmannstr. 8

Post Office Address:

City: Rostock State: Zip: D-18055 Country: Germany Applicant Authority:

☐ Additional inventors are being named on supplemental sheet(s) attached hereto